the binding so measured in insulin-treated diabetics appears to be specific, for the addition of stable insulin reduces the amount of labelled insulin bound. The fairly close correlation between ethanol and immunoprecipitation results (with an antihuman gammaglobulin) identifies the binding protein as being mainly gammaglobulin; though antibodies have been detected occasionally in the B₂A and B₂M globulins (Yagi *et al.*, 1963), these would also be precipitated by ethanol.

The ethanol method can be used by any hospital equipped with centrifuge and counting facilities for ¹³¹I or ¹²⁵I; the equipment and technical skill required are minimal, and the method has the advantage of considerable simplicity compared with currently established techniques. It has practical application in various circumstances. It can identify when severe chronic insulin resistance depends on excessive antibody formation, and it can assess the effectiveness of therapy in such patients. Evidence for chronic insulin administration can also be obtained, as when repeated "spontaneous" hypoglycaemia is suspected of being due to self-administration of insulin.

Summary

After incubation of serum with ¹³¹I-insulin, ethanol added to a final concentration of 80% precipitates protein-bound ¹³¹I-insulin. With diluted sera the method gives a useful index of

the titre of insulin antibodies present, and is in good agreement with a more difficult immunoprecipitation technique. Chronic insulin resistance in diabetes may conveniently be assessed by this procedure.

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Osteomyelitis: An Unusual Sequel to Neutropenia*

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Bacterial infections often complicate neutropenia (*Brit. med. J.*, 1964). It is surprising, therefore, that bacterial osteomyelitis does not seem to have been previously reported as a sequel to neutropenia in adults.

We wish to report the occurrence of osteomyelitis in two adult patients with neutropenia, both of whom had been treated with corticosteroids, and a third patient in whom neutropenia may have been responsible for increased susceptibility to bone infection.

In each case the clinical presentation and course of the infection were unusual and radiology was of importance in establishing the diagnosis.

Case 1

A 43-year-old Iranian was first seen at Hammersmith Hospital in August 1964, when he gave a three-month history of malaise, shortness of breath, and spontaneous bruising. Clinical examination showed nothing abnormal apart from pallor and bruising. The white cell count was 64,000/cu. mm. with 40% blast forms, and sternal marrow biopsy showed a heavy infiltration with blast cells. A diagnosis of acute lymphoblastic leukaemia was made and treatment begun with prednisone, 40 mg./day. Within five weeks the peripheral blood and the marrow had become normal. Maintenance therapy with mercaptopurine 150 mg./day was then started, being continued in Iran until June 1965, when the dose was reduced to 50 mg./day. In July a further bone-marrow biopsy revealed relapse of the leukaemia. Prednisone 40 mg./day was given, together with

vincristine (two doses of 2 mg. intravenously). A second remission rapidly resulted, and the prednisone was tailed off. Early in December he again became ill, and prednisone 40 mg./day was reinstituted. A week later he complained of swelling and tenderness of both shins.

On 28 December he was readmitted to the Hammersmith Hospital. His temperature was 99.6° F. (37.6° C.). Examination revealed tenderness with redness and swelling over the lower third of the left tibia, and oedema of the left ankle. Small haemorrhages were present on the soft palate, left conjunctiva, and left retina.

Investigations.—Haemoglobin 11.2 g./100 ml.; white cell count 2,000/cu. mm. (84% lymphocytes, occasional blast cells, neutrophils 300/cu. mm.); platelets 10,000/cu. mm.; E.S.R. greater than 100 mm./hour. Bone-marrow aspiration: repeated dry taps. Serum uric acid 5 mg./100 ml. Haemoglobin electrophoresis normal. Widal reaction negative. Brucella agglutinins negative. Immuno-electrophoresis; γ M component 320 mg./100 ml.

He was maintained on prednisone 40 mg./day and also given intravenous vincristine (6 mg. in three weeks). The white cell and platelet counts rapidly returned to normal. The course of the leukaemia during the next six months and its relation to treatment is shown in Fig. 1.

Three days after admission effusions developed into both knee joints, accompanied by a pyrexia of 104° F. (40° C.). Two successive blood cultures grew β -haemolytic streptococci sensitive to ampicillin and cloxacillin. Treatment was begun with these antibiotics with temporary resolution of fever and disappearance of the effusion from the left knee, but not the right. Fever recurred and persisted in spite of treatment with a variety of antibiotics. The temperature chart and its relation to antibiotic therapy are shown in Fig. 2. The right knee joint was aspirated on 10 occasions. Purulent fluid was obtained repeatedly, but aerobic and anaerobic

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cultures were invariably sterile, and acid-fast bacilli and fungi were neither seen nor grown. Twelve blood cultures over the course of the illness were all negative. Pain and swelling on the left shin increased and persisted.

Radiographs of both the right knee and the left tibia on 30 December showed no abnormality. By 1 February 1966 patchy bone destruction with overlying periosteal reaction had appeared on the

medial aspect of the lower left tibial shaft (Fig. 3). At this time x-ray films of the right knee were still interpreted as normal.

On 28 February open biopsy of the left tibia was performed (Mr. W. H. Stephenson) with removal of necrotic bone and small amounts of pus, both of which were sterile. Histologically there was thickening of the periosteum and heavy infiltration of both periosteum and fibrous tissue surrounding cortical bone with chronic inflammatory cells. The appearances were those of chronic osteomyelitis. There was no evidence of leukaemic infiltration (Professor C. V. Harrison).

Radiographs of the right knee on 14 March showed slight demineralization of the lower femur with a possible lytic area above the lateral femoral condyle. Tomographic examination revealed an extensive lytic lesion of the inner bone, with sclerosis around it, but with no periosteal reaction. Further radiological examination of the left tibia on 30 April showed an extension of the bone resorption and an increase in the periosteal new bone formation. By 9 June a long, dense sequestrum had appeared on the lateral aspect

FIG. 1.—Case 1 (acute lymphoblastic leukaemia). Haematological findings and treatment from December 1965 until death. Note inadequate neutrophil response to infection.

FIG. 2.—Case 1. Average daily temperature and antibiotic therapy from December 1965 until death. Note fever responded very slowly to antibiotic therapy. AMP=Ampicillin 500 mg. q.d.s. (oral). CLO=Cloxacillin 500 mg. q.d.s. (oral). GEN=Gentamicin 80 mg. t.d.s. (parenteral). KAN=Kanamycin 50 mg. q.d.s. (parenteral). LINCO=Lincomycin 250 mg. q.d.s. (oral).

FIG. 3

FIG. 4

FIG. 5

FIG. 3.—Case 1. Left tibia. 1 February 1966. Arrow points to evidence of cortical bone destruction and minimal periosteal reaction. FIG. 4.—Case 1. Left tibia. 9 June 1966. There is a large cavity within the bone, associated with extensive periosteal reaction and a long dense cortical sequestrum. FIG. 5.—Case 1. Right knee. 3 July 1966. Patchy sclerosis in the lower femoral condyle and upper tibia with areas of lysis; a periosteal reaction has appeared on the medial aspect of the lower femur.

of the tibial shaft (Fig. 4). On 6 June a periosteal reaction was noted above the medial femoral condyle on the right side, and by 3 July this, together with the other changes, had increased (Fig. 5).

In spite of radiological progression of the lesions, clinical improvement occurred with antibiotics, immobilization of the right knee, and drainage of the left tibia. By May 1966 no effusion was detectable and fever had largely subsided. Mobilization was begun. The leukaemia remained under adequate control from mid-January to early June 1966 (Fig. 3), but then peripheral blood and bone-marrow relapse occurred. Treatment with vincristine produced no response, and large doses of intravenous methotrexate were employed (120 mg. in 36 hours). The patient subsequently developed a *Pseudomonas pyocaneae* septicaemia, from which he died on 22 July.

Post-mortem examination of the right femoral condyles and left tibial shaft showed areas of softening and bone necrosis. Microscopical appearances were of chronic osteomyelitis with periosteal thickening, scattered necrosis of fat and bone, marrow fibrosis, and infiltration with plasma cells containing Russell bodies. A heavy growth of *Ps. pyocaneae* was obtained on post-mortem culture of bone and marrow from both tibia and femur.

A full post-mortem examination was not possible.

Case 2

A 41-year-old Egyptian had received intermittent treatment with chloramphenicol for chronic prostatitis. In August 1965 he developed anaemia and subcutaneous haemorrhages. Peripheral blood

FIG. 6.—Case 2. Left ankle. 3 May 1966. A periosteal reaction is present along the medial side of the lower tibial shaft and a radiolucent cavity is just visible within the bone.

examination showed pancytopenia (haemoglobin 10.6 g./100 ml., total white cell count 1,300/cu. mm. (neutrophils 1,000/cu. mm.), platelets 12,000/cu. mm.), and the bone marrow was found to be hypoplastic. He was treated with blood transfusions, prednisone, erythromycin, and ampicillin. In February 1966 he developed diabetes, requiring insulin therapy, which resolved rapidly when prednisone was tailed off. On 20 April he was admitted to Hammersmith Hospital for further assessment of his blood disorder. His only complaint was of slight swelling and tenderness over the left shin, the onset of which had coincided with the reduction in steroid therapy, at which time the neutrophil count was 1,600/cu. mm. Examination revealed slight tenderness over the lower third of the left tibia and moderate oedema of the left ankle. Temperature was normal.

Investigations.—Haemoglobin 12.4 g./100 ml.; reticulocytes 2%; platelets 86,000/cu. mm.; white cell count 7,000/cu. mm. (neutrophils 2,800/cu. mm.). Marrow biopsy: normal apart from slight hypocellularity. E.S.R. repeatedly raised over 100 mm./hour. Mid-stream specimen of urine contained moderate numbers of pus cells with a significant growth on two occasions of *Escherichia*

coli and once of *Klebsiella*. Serum uric acid 7 mg./100 ml. Urea and electrolytes, serum bilirubin, acid, and alkaline phosphatase normal. Serum albumin 4.4 g., serum globulin 2.9 g., electrophoretic strip normal. W.R., Reiter, and G.C.F.T. were negative. Three samples of sputum were negative for acid-fast bacilli. Three blood cultures were sterile. Radiological examination of the left tibia on 3 May showed a periosteal reaction along the medial cortex associated with a large cavity in the lower part of the bone (Fig. 6). This was confirmed by tomography. Open biopsy (Mr. N. Reis) showed soft necrotic bone which was curetted out. On histological examination the trabecular bone showed increased amounts of fibrous tissue containing small pieces of dead bone and foci of chronic inflammation, the inflammatory cells being largely histiocytes and plasma cells. In some areas small groups of polymorphs and a few bacterial clumps were seen. Three fragments of bone were sent for culture. From one a light growth of *E. coli* was obtained, and from another a light growth of *Klebsiella*. The third culture was sterile. The patient was treated with cephaloridine, 500 mg. intramuscularly six-hourly for six weeks. A repeat x-ray examination on 23 June showed no evidence of progression of the bone lesion.

Case 3

A woman, now aged 68, was admitted to Lewisham Hospital in December 1963. She was found to be anaemic, owing to paroxysmal nocturnal haemoglobinuria, and an incidental finding was the presence of haemoglobin J_{Baltimore}. These findings have been described by Holman *et al.* (1964). Before and after this admission she received intermittent courses of antibiotics for chronic bronchitis. In 1964 she developed intermittent pain in the right lower thigh and knee, which became continuous by November 1965. Biopsies of the medial aspect of the right lower femur were taken in August 1964 (Lewisham Hospital) and March 1965 (Ashford

FIG. 7.—Case 3. Right femur. 31 December 1965. Massive sclerosis in and around the lower femoral shaft associated with areas of lysis and a periosteal reaction of long standing.

Hospital). The slides have been reviewed (Professor C. V. Harrison). Histological appearances of both specimens were similar and showed periosteal thickening with recently formed woven bone showing osteoblastic activity beneath it. Very few inflammatory cells were present. The appearances are thought to be consistent with low-grade infection.

In November 1965 she was admitted to Hammersmith Hospital for reassessment in view of increasing transfusion requirements. Examination revealed a thin, anxious woman with signs of chronic bronchitis. The spleen was palpable 8 cm. below the left costal margin and the liver just palpable. There was marked wasting of the right quadriceps muscle and tenderness over the entire right lower femur. The knee was hot, swollen, and tender, with severe limitation of movement due to pain.

Investigations.—Haemoglobin 7.5 g./100 ml.; reticulocytes 11.4%; M.C.H.C. 30%; white cell count 11,000/cu. mm. (neutrophils 80%); platelets 176,000/cu. mm.; E.S.R. persistently between 60 and 150 mm./hour. Haemolysis in unacidified serum 1.8%, in acidified serum 12.2%. Serum iron 74 µg./100 ml. Urine haemosiderin present. Marrow examination showed active leucopoiesis with some hypersegmentation of neutrophils and giant metamyelocytes. Serum albumin 4.5 g./100 ml., serum globulin 2.3 g./100 ml., serum alkaline phosphatase 13 K.A. units. Midstream specimen of urine: moderate pyuria with heavy infection of *E. coli*.

An x-ray film of the right femur revealed a patchy area of sclerosis and lysis in the lower femoral shaft, with a dense periosteal new bone formation around it (Fig. 7). She was transfused with 9 units of washed cells and discharged.

She was readmitted to Hammersmith Hospital on 14 December 1965 with a recrudescence of anaemia. Bone pain was more severe, and shortly after admission a large effusion developed in the right knee joint. Aspiration yielded 60 ml of straw-coloured fluid containing 60 white cells/cu. mm. (53% neutrophils), 3.6 g. protein/100 ml., and 115 mg. glucose/100 ml.; no urate crystals were visible. Culture, both aerobic and anaerobic, was sterile. No acid-fast bacilli or fungi were cultured. Mild intermittent fever was present during this admission.

Further Investigations.—Total white cell count 3,000/cu. mm. (neutrophils 82%); platelets 136,000/cu. mm. Blood cultures sterile. Widal reaction negative. Brucella agglutinins negative. Serum antistaphylococcal alpha-haemolysin 0.4 unit/ml. (low normal range). X-ray film right femur: no change.

Discussion

A first attack of haematogenous osteomyelitis is rare after the cessation of growth (Trueta, 1953). When osteomyelitis occurs in adults it is usually associated with a predisposing cause such as local trauma, soft-tissue infection, or diabetes mellitus.

Bacterial osteomyelitis in blood disorders in which neutropenia occurs is, surprisingly, not mentioned in the literature. Hersh *et al.* (1965) record one case of mucor osteomyelitis and none of bacterial osteomyelitis in 414 deaths due to acute leukaemia, though infection was a major cause of death in 70%. Crosby's (1953) review of paroxysmal nocturnal haemoglobinuria does not refer to osteomyelitis. No cases of osteomyelitis are reported by Scott *et al.* (1958), Mohler and Leavell (1958), or Lewis (1965) in their reviews of aplastic anaemia.

The one factor in common with the three cases described was an inadequate neutrophil response to infection. In Cases 1 and 2 the onset of symptoms occurred when there was an absolute neutropenia. In Case 3 worsening of symptoms coincided with a fall in total white cell count from 11,000 to 3,000/cu. mm. In paroxysmal nocturnal haemoglobinuria a failure of normal leucocyte response to infection (Dausset *et al.*, 1956) and reduced phagocytic ability of the neutrophils (Penny and Galton, 1966) are documented.

Cases 1 and 2 were receiving systemic steroid therapy when symptoms began, and it may be that this contributed to an increased susceptibility to infection. However, skeletal infections in patients on systemic steroid therapy are generally of extremely rapid and often silent progression, with extensive bone destruction and little bone reaction (Murray, 1960). Our two patients do not fit these criteria.

There was no evidence of a failure of immune response to infection in any patient. In particular, none had hypogammaglobulinaemia, and in Case 1 the immune globulin level was raised.

Clinical features of osteomyelitis in these cases were unusual. Symptoms were slow in onset and of mild or moderate severity, and progress of the disease was indolent. All three patients were ambulant on admission to hospital. Clinical and radiographic similarities exist between these cases and those of "primary subacute pyogenic osteomyelitis" in East Africa described by Harris and Kirkaldy-Willis (1965). These authors attribute the clinical manifestations to a decreased virulence of the infecting organism associated with an increase in host resistance.

In Case 1 β -haemolytic streptococcal septicaemia was demonstrated, and it may be that bone infection was initially with streptococci, pseudomonas septicaemia occurring as a terminal event. In Case 2 *E. coli* and klebsiellae were cultured from bone and urine. The blood disorders concerned predispose to such unusual infections (Mohler and Leavell, 1958; Hersh *et al.*, 1965), and it seems likely that the unusual clinical features of osteomyelitis were determined by the unusual nature of the infecting organisms. Inadequate host resistance was a feature of our patients, in contrast with those of Harris and Kirkaldy-Willis (1965).

A further possible modifying factor is antibiotic therapy received at the onset of infection (Cases 2 and 3) or subsequently (Case 1).

Owing to the unusual clinical features in these patients, radiology assumed increased importance in diagnosis. In Case 2, whose clinical signs were minimal, it is possible that the diagnosis of osteomyelitis would not have been made had not experience of Case 1 prompted radiological examination.

Both the value and the deficiency of x-ray examination are of interest. Great support for the diagnosis of osteomyelitis was obtained from the typical x-ray appearances of the lesions in the three patients. It should be noted that the first x-ray examination of the tibia in Case 1 showed no bone lesion at all. However, it is well known that bone changes do not usually appear on a radiograph until at least 10 days after the onset of acute osteomyelitis.

We feel that all patients with any blood disease likely to lower or impair neutrophil response should undergo x-ray examination of any painful bones, and we stress that if the x-ray films are negative at the first examination they should be repeated after an interval of two to four weeks.

Summary

Three patients with osteomyelitis complicating failure of normal neutrophil response to infection are described. Pathogenesis, clinical features, and diagnosis are discussed. The value of x-rays in diagnosis is emphasized.

We wish to thank Dr. V. H. Bowers, Professor L. P. Garrod, Dr. D. A. G. Galton, Professor C. V. Harrison, Mr. N. Reis, and Mr. W. H. Stephenson for their help in the study of these patients.

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